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Upon the Number of Micro-Organisms in the Air of Winnipeg.

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IV.—*Upon the Number of Micro-Organisms in the Air of Winnipeg.*

By A. H. R. BULLER, D.Sc., F.R.S.C., and CHAS. W. LOWE.

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INTRODUCTION.

The authors have determined the number of micro-organisms present in the air of Winnipeg during every week throughout a whole year. Hitherto, no statistics of this kind have been compiled for Central Canada, and it was thought that the investigation might possibly have some bearing on the spread of certain diseases in addition to its general biological interest.

As long ago as 1887 Frankland[1] published a curve showing the seasonal variation in the number of micro-organisms in the air of London. The English climate, as is well known, is very humid; and the winters are usually wet and mild. In contrast with this, the climate at Winnipeg on the whole is very dry, especially during the winter. Whereas in England snow covers the ground for only a few weeks in the year, at Winnipeg the ground is frost-bound and covered with snow for something like five months. The difference in the climate of the two places, therefore, is very marked; and, as we shall see, a comparison of Frankland's observations with those made by us shows how greatly the distribution of micro-organisms is affected by climatic conditions.

Those who live in Manitoba are aware how very free the air is from dust particles during the winter months. On account of the dustlessness of the atmosphere, it was expected before the investigations began that, at this period of the year, there would be a very small number of micro-organisms in the air of Winnipeg relatively to that of London as determined by Frankland. This expectation has been fully realised; and for healthiness, so far as the number of micro-organisms is concerned, the climate of Central Canada during the winter must be one of the best in any civilised country in the world.

Among earlier workers upon the distribution of micro-organisms in the air may be mentioned Pasteur, Miquel, Hansen, Fischer, Moreu & Miquel, Minervini, Giacosa, Miflet, Selander, Cacace, Shibuya, and Frankland. Recently, Saito,[2] using the plate method, has carried out

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[1] P. F. Frankland. Further Experiments on the Distribution of Micro-organisms in Air (by Hesse's Method). Proc. of the Roy. Soc., Vol. 42, 1887, pp. 267–282.

[2] K. Saito. Untersuchungen über die atmosphärischen Pilzkeime, The Journal of the College of Science, University of Tokyo, Vol. xxxvii, 1904, pp. 1–58.

a series of experiments at Tōkyō throughout a whole year, and we refer the reader to his paper for the literature of the subject.

Methods.

Two methods have been employed: (1) the volumetric, and (2) the plate method; and parallel determinations have been made weekly, without any break, during the whole of one year. This is the first time that parallel observations by these two methods have been carried on during so long a period of time. The curves given by the results are not identical in form, and a study of them shows that variations in external conditions do not result in parallel variations in the number of micro-organisms given by the two methods. Thus, for instance, a strong wind increases the number of micro-organisms settling upon an exposed plate to a much greater relative extent than it increases the number in 10 litres of air.

The Volumetric Method.—The volumetric method is practically that devised by Professor Percy Frankland[1] for his experiments in England. However, for the experiments carried out on the campus of the university of Manitoba, it was found convenient to substitute a ten-litre aspirator for a pump, in order to draw the air through the collecting tubes. To this extent Frankland's method has been simplified and rendered much easier in carrying out, more especially during the great cold of the winter months and during those weeks in summer when mosquitoes are troublesome.

The essential parts of the apparatus were as follows: (1) two collecting tubes, which we shall term the experimental and control tubes respectively; (2) an aspirator capable of aspirating 10 litres of air through the experimental tube; and (3) a piece of lead piping ten feet long, by means of which the experimental tube was connected with the aspirator.

When the aspirator was set up on the University campus, it had the appearance shown in Fig. 1. The experimental and control tubes were attached to a clamp-stand at a height of about two feet from the ground, and the aspirator was supported on a stool.

The experimental tube (Fig. 2, A) was constructed from a glass tube which was 5 inches long and had an internal diameter of 0.25 inches. It was heated in a flame so as to make a constriction (Fig. 2, *c*), about 1.25 inches from one end. Some glass wool was pushed down the tube with the help of a small glass rod, so as to form a layer on one

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[1] P. F. Frankland. A new method for the quantitative estimation of the micro-organisms present in the atmosphere. Phil. Trans. Roy. Soc., Vol. 178 (1887) B, pp. 113–152.

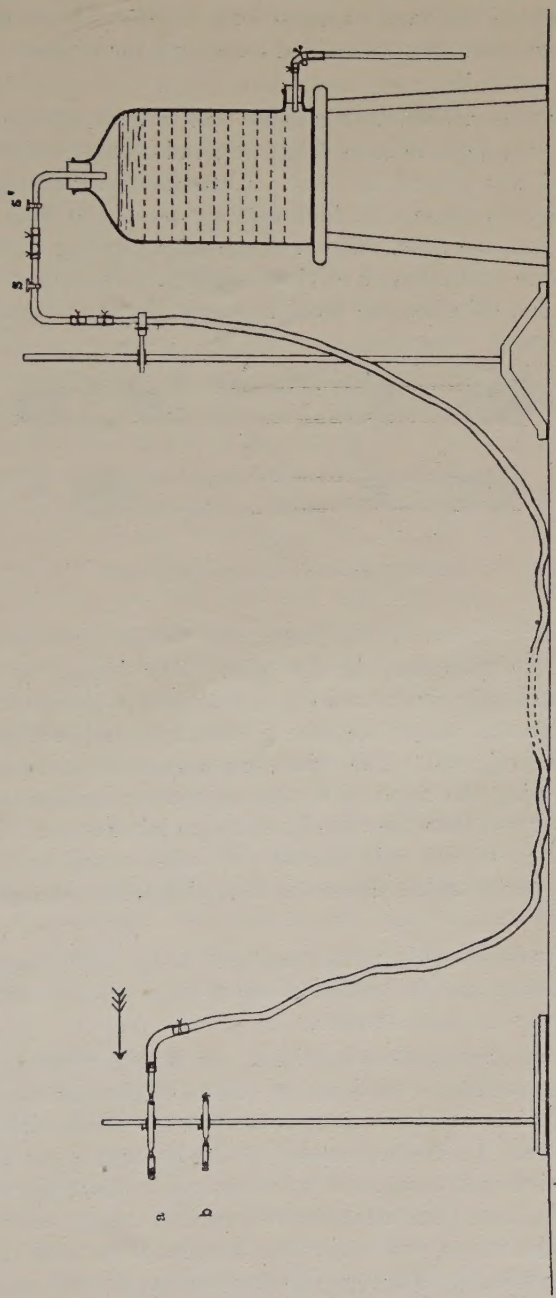

Fig. 1.

side of the constriction. Next, some finely powdered cane-sugar was introduced above the wool so as to form a second layer of about the same thickness, and then was added another layer of glass wool similar to the first.

Another constriction, similar to the first, was then made 1.25 inches from the other end of the tube (Fig. 2, *d*), and a plug composed entirely of glass wool was placed in front. Immediately after the first constriction had been made and, therefore, previously to the introduction of the sugar into the tube, the end of the tube *f* in Fig. 2, was rounded off in the flame and allowed to close slightly. The other end, *a*, was usually rounded off after the second constriction had been made.

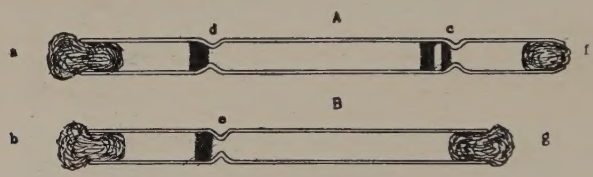

Fig. 2.

REDUCED TO ONE HALF ORIGINAL SIZE.

After the insertion of the plugs, two cotton wool stoppers were pushed into the open ends of the tube. The stopper at the end of the tube at which the air entered (*a*) was allowed to protrude somewhat, but that at the exit end of the tube (*f*) was trimmed in a flame so that no free hairs projected. The trimming prevented the stopper being pulled out during the removal of the connecting rubber tubing when it was necessary to take the tube back to the laboratory. The slightly closed condition of the exit end of the tube served to prevent the stopper from being sucked down the lead pipe whilst the air was being aspirated.

The control tube (Fig. 2, B) was made in the same way as the experimental except that it contained but a single plug of glass wool (*e*) and was usually one inch shorter.

The aspirator is shown in Fig. 1. It had a capacity of twelve litres and was graduated in litres by means of circular markings. At the top and bottom of the aspirator were holes fitted with perforated rubber stoppers. In the perforation of the lower stopper a glass tube was fixed which was connected by means of a short piece of rubber tubing with a long glass tube which reached nearly to the ground. The exit of the water was controlled by means of a clamp attached to the rubber tubing. Through the perforation of the upper stopper there projected one arm of a U-shaped piece of glass tubing provided with two stopcocks (s and s^1).

Before an experiment was to take place, the aspirator was filled up with water, set upright on its stand, and the level of the water allowed to descend to the first circular marking. Since nearly half of the experiments had to be carried out when the temperature of the air was below freezing point, and many when the temperature was below zero Fahrenheit, it was necessary under these conditions to fill the aspirator with warm water.

Owing to the extreme paucity of micro-organisms present in the air during the winter, it was found advisable very frequently to aspirate from 20 to 100 litres of air through the experimental tube, instead of merely 10 litres. When more than 10 litres was to be aspirated, a second aspirator, similar to the first, was prepared. As soon as 10 litres had been aspirated by the first aspirator, the stopcock, *s*, was closed and the stopper of the stopcock, s^1, was pulled out. The glass tube passing through the stopper at the top of the aspirator was withdrawn and the second aspirator substituted for the first, and connected in a similar mannner. The water in the fresh aspirator was allowed to descend to the first circular marking. The stopper of the stopcock, s^1, was then replaced, and when both of the glass stopcocks had been opened and the clamp from the exit tube had been removed, a further 10 litres could be aspirated through the experimental tube. The aspiration of 100 litres of air involved nine changes of the aspirators. Each change was carried out in about half a minute. The aspiration of 10 litres was accomplished in 12 minutes.

The flexible lead pipe, which was ten feet long and which had an internal diameter of a quarter of an inch, was connected with the experimental tube and the aspirator by short lengths of rubber tubing. The object in using the lead pipe was to allow of the air being aspirated through the experimental tube at a distance of about ten feet from the aspirator, which it was necessary for the experimenter to manipulate.

The apparatus was so arranged during the aspiration of the air that the wind always moved in a direction parallel to the axis of the experimental tube and from its exit to its entrance end. The direction of the wind is indicated in the figure by means of an arrow. The lead pipe was placed at right angles to the direction of the wind, so that any micro-organisms disturbed by the manipulation of the aspirator could not pass to the mouth of the experimental tube. The advantage of using tubing made of lead rather than of glass or rubber lies in the fact that a lead tube is sufficiently flexible but, at the same time, not readily cracked or broken.

A series of volumetric experiments was made at St. Charles, at a distance of six miles from the city of Winnipeg. Here, on the open prairie, it was not found convenient to use the aspirator, owing to the

difficulty of obtaining water. A pump like that used by Frankland was therefore employed. The apparatus, when set up with the pump included, is shown in Fig. 3. The pump, which was made of brass, was provided with two taps, the entrance (A) and the exit (B). The former was attached to the experimental tube by means of the lead pipe, and a mercury manometer was fitted up in the position shown in the figure. The capacity of the pump was carefully measured in the laboratory; it was found that 55 strokes of the pump were necessary in order to draw 10 litres of air through the experimental tube. The air was drawn through the tube in the following manner. The entrance tap A was closed and the exit tap B was opened, whereupon the handle of the pump was pushed down as far as it would go. Next, the tap A was opened and the tap B closed; the handle of the pump was then pulled out to its extremity, and by this means a definite volume of air was drawn through the experimental tube. As the handle of the pump was drawn upwards, the mercury rose in the manometer. The fall of the mercury in the manometer arm to the level of the mercury in the glass beaker—a process which was watched by means of a mirror inclined at an angle of 45° to the ground—served to indicate that the air in the pump stood at atmospheric pressure. When this was observed, the tap A was closed, the tap B opened, and the handle of the pump was pushed downward in preparation for another stroke. The time required for 55 strokes, which were necessary in order to draw 10 litres of air through the experimental tube, was about 25 minutes. With the aspirator, as we have seen, 10 litres could be drawn through in about 10 minutes, so that when the pump was used, the air passed through the experimental tube at somewhat less than one-half the speed it did when the aspirator was employed. The great advantage of the pump over the aspirator is its ready portability.

The sterilization and transportation of the experimental and control tubes were carried out as follows. The two tubes were each placed in a test tube closed with cotton wool stoppers. In order to prepare additional sterilised stoppers for the experimental and control tubes, for use after the aspiration of the air through the former, a short glass tube, similar to that used for the experimental and control tubes, was fitted up with cotton wool stoppers with small bits of a wooden or glass rod as cores. This tube was placed in a third stoppered test tube.

The three tubes, with their contents, were sterilised by placing them in a dry steriliser at a temperature of 130° C. for three hours. The test tube contents enclosed by the test tubes could readily be transported to the place where the experiment was to be made without any fear of contamination.

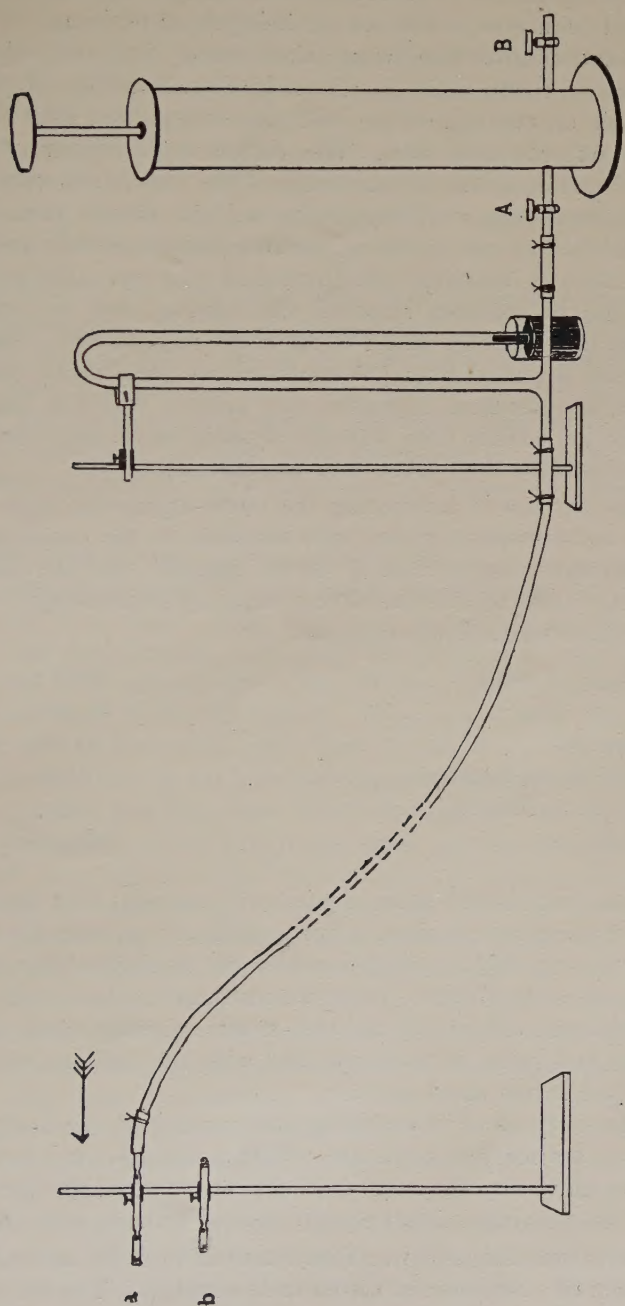

Fig. 3.

When it was desired to make an experiment, the aspirator or pump and the lead tubing were first set in their proper positions. The experimental and control tubes were taken out of their test tubes and attached to the clamp stand as shown in Figs. 1 and 3. A piece of rubber tubing on one end of the lead pipe was pushed over the exit end of the experimental tube. The cotton wool stoppers, *a* and *b* in Figs. 1, 2 and 3, at the entrance ends of the two tubes, were pulled out and thrown away, whereupon the air was drawn through the experimental tube by one or other of the two methods already described. As soon as this had been done, the third glass tube was taken out of its test tube and its stoppers removed and inserted into the entrance ends of the experimental and control tubes respectively. The lead pipe was then detached from the experimental tube which was then replaced in the sterilised stoppered test tube in which it had been transported; the control tube was also replaced in its test tube. The two test tubes were then taken into the laboratory.

For the purpose of cultivating the micro-organisms, three flasks, fitted with cotton-wool stoppers, were sterilised in the usual manner. One of them was a conical flask of 100 c.c. capacity, and the other two were Florence flasks of 250 and 500 c.c. capacity respectively.

The culture medium was composed of:

Distilled water	1000 c.c.
Grape sugar	10 gm.
Peptone	10 gm.
Liebig meat extract	3 gm.
Sodium chloride	5 gm.
Gelatine	100 gm.

This medium, before being cleared with an egg, was neutralised in as careful manner as possible, as our preliminary experiments showed us that even very slight acidity considerably diminished the number of colonies which developed. After the medium had been well mixed, neutralization was effected by the well-known phenolphtalein method. Fairly large test tubes were nearly filled with the medium, which was then sterilised in the usual manner.

For the purpose of determining the number of micro-organisms in the plugs, the medium contained within a test tube was melted by means of warm water; about one-half of it was poured into the largest flask and the remainder about equally divided between the other two.

The experimental tube was then removed from its test tube and, with the help of a file, broken across in the middle. The cotton wool stopper at the entrance end of the tube (Fig. 2, *a*) was then removed and inserted into the newly-made open end of the exit half of the tube,

and this half was laid on a sterilised surface. The glass wool plug, *d*, in the entrance half of the tube was then pushed by means of a sterilised glass ramrod into the largest of the flasks, which was again closed by its cotton wool stopper which had been temporarily removed. The exit half of the tube was then taken up, its cotton wool stoppers removed, and its plug of glass wool and sugar, *c*, pushed down into the medium-sized flask. The control tube was then removed from its test tube, its cotton wool stoppers taken out and its plug, *e*, pushed down into the small conical flask.

By shaking the three flasks, the plugs introduced into each became disintegrated and the constituent parts uniformly distributed in the still fluid medium. By rotating or otherwise manipulating the flasks under a stream of water, the gelatine in each was caused to solidify and become evenly spread over their bases and sides. When this had been done, the flasks were labelled and set in a dark cupboard in a warm laboratory, the temperature of which was usually about 17° C.

After three or four days' incubation, colonies of bacteria and yeast and the mycelia of moulds became visible; and their number was counted at the end of a week. The first plug, *d*, taken from the entrance end of the experimental tube gave the number of micro-organisms which had been collected from the 10 litres or other quantity of air which had been drawn through it. The second plug, *c*, taken from the exit end of the experimental tube served as a control for the first plug. It was argued that if any micro-organisms had failed to be collected in the first plug, they would be caught in the second, if not in the glass wool, at any rate in the finely powdered sugar. Only on very rare occasions were any micro-organisms developed in the flask containing the second plug, *c*, and not once did their number exceed three. On the single occasion when three were obtained in this plug, the maximum number of micro-organisms, namely 260, were developed from the first plug. It is clear from this that the first plug successfully arrested practically all the micro-organisms contained in the air drawn through it. When micro-organisms were developed from the second plug, their number was added to those developing from the first. The plug of the control tube (Fig. 2, *e*) served to show whether or not the experimental and control tubes had been properly sterilised in the first place, and whether or not any micro-organisms had settled in the mouth of the two tubes through the agency of the wind or air currents during the time that the cotton wool stoppers had been removed from the entrance ends of the two tubes. It was only exceedingly rarely that micro-organisms developed in the flask containing the plug of the control tube, and on no occasion did their number

exceed two. When an occasional micro-organism did develop, the contamination could be traced to a change in the direction of the wind during the exposure of the tubes in the field. When micro-organisms developed from this control plug, their number was counted and an equal number was deducted from the number which developed in the flask containing the first plug (*d*) of the experimental tube.

The Plate Method.—Shallow Petri dishes, five and a half inches wide and half an inch high, were sterilised and then partially filled with the culture medium in the usual manner. When this had solidified, the plates were enclosed in a sterilised glass vessel, to protect them during their transportation to the field. When the plates had been brought to the place of exposure, they were set on a horizontal support raised from three to six inches above the level of the ground which, it may be remarked, is exceedingly flat everywhere in the neighbourhood of Winnipeg. The lids of the Petri dishes were raised and the surface of the gelatine exposed to the atmosphere for one or more minutes. The time of exposure varied considerably: at those times when micro-organisms were abundant, *i.e.*, during the summer, and when the wind was strong, it was only one minute in length; but after heavy rain and during the winter months, when the air was almost sterile, owing to the exceeding paucity of the micro-organisms present, it was found necessary, in order to collect any micro-organisms at all, to increase the time of exposure to 5 minutes, 10 minutes, or, on some occasions, to one hour. After the exposure, the lid of the Petri dish was replaced; the closed lid was again covered with the sterilised glass vessel and then taken back to the laboratory. The Petri dishes were kept in the same room, and therefore subjected to practically the same conditions, as the flasks containing the plugs.

The counting of the colonies and fungus mycelia was usually carried out on the fourth day after making the cultures. It was always found that micro-organisms on the surface of the gelatine developed more rapidly than those which were embedded in the gelatine in the flasks. Doubtless, this difference in the rate of development is to be accounted for by the difference in the accessibility to oxygen for the micro-organisms in the two cases. In order to count the number of colonies, etc., which had developed, the plate was placed on a piece of black paper divided into small areas by white lines. The number in each area was counted in succession. The total was thus obtained without any danger of any colonies, etc., being overlooked or counted twice. The unit of measurement fixed upon for the rate of fall of micro-organisms was the number falling on one square foot per minute, and the results of the plate records have been tabulated according to this standard. The actual area of the gelatine exposed was 20.95 square inches.

RESULTS.

The results of the experiments are given in the appended Table. The first column gives the date of each experiment, the second the number of micro-organisms in 10 litres of air, the third the number falling per square foot per minute, the fourth the ratio of the number falling per square foot per minute to the number in 10 litres of air, the fifth the temperature at the time of the experiment in degrees Centigrade, and the sixth remarks upon the weather. Four degrees of wind velocity were recognised: slight, moderate, strong, and gale.

Date of Experiment	Number in 10 litres	Number falling per sq. foot per min.	Ratio of the number falling per sq. foot per min. to the number in 10 litres	Temperature in degrees Centigrade	Remarks upon the weather
1908					
April 6....	6	341	51	—6	Wind N.E., strong
" 9....	..	385	..	+2.5	" S.W., stronger
" 10....	..	4,180	..	+4	" N.N.W., gale
" 11....	..	436	..	13	" S,. moderate
" 13....	30	8,506	283	0.0	" N., gale
" 20....	22.5	407	13.6	10	" E., slight
" 27....	0.0	206	..	2	" N., strong, rain on 24th and 25th, snow on 26th
" 28....	—	694	....	3	Wind, N., moderate
" 29....	15	124	8.2	4.5	" N.W., slight
" 30....	..	1,839	....	6.5	" N.W., moderate
May 1....	25	821	32.7	3	" N.W., strong
" 2....	..	1,641	....	5.5	" N.W., slight
" 3....	..	2,394	....	13	" N.E., slight
" 4....	13	1,205	92.7	11	" N.E., slight
" 11....	37	2,304	62.1	15.5	" N.E., moderate, experiment made immediately before rain
" 18....	15	1,551	13.4	22.5	" S.W., strong, showery since 11th
" 23....	16	247	16.5	18	Wind, S., moderate, rain from morning of 11th to 22nd
" 29....	46	1,529	33.2	20.5	Wind, N., moderate
June 1....	40	203	5.07	25	" S.E., moderate
" 8....	19	527	22.7	8.5	" N.,strong, frosts on 6th and 7th

Date of experiment	Number in 10 litres	Number falling per sq. foot per min.	Ratio of the number falling per sq. foot per min. to the number in 10 litres	Temperature in degrees Centigrade	Remarks upon the weather
June 15....	25	545	21.8	15	Wind, S.E., moderate, cold week, frosts until 13th
" 22....	26	363	13.91	19	Wind, N., slight, heavy rain on 20th, less on 21st
" 26....	37	2,085	56.4	24	Wind, S., strong
" 29....	10	189	18.9	15	" N., slight, rain on 28th and early morning of 29th
July 6....	18	414	23	17	Wind, N., slight
" 13....	45	1,446	32.6	21	" N.W., moderate
" 20....	53	1,599	30.1	25	" N.W., moderate
" 27....	31	158	5.09	29	" S,. slight, after several hot days with very little wind
Aug. 3....	62	407	6.5	22	Wind, N.W., strong
" 10....	46	1,519	33	25	" W., strong
" 17....	38	846	22.4	22	" S.W., moderate
" 24....	137	988	7.2	20	" N., moderate
" 31....	12.5	312	24	19	" S.E., moderate, showery, 29th and 30th heavy rains
Sept. 7....	30	2,732	91	21	Wind, S.W., strong
" 14....	20	676	33.8	24	" S.E., moderate
" 21....	100	777	7.77	20.5	" S.E., moderate
" 28....	263	763	2.9	0.5	" N.W., moderate, warm rain from 24th to 26th, frosts on nights of 1st & 2nd
Oct. 5....	21	254	12.09	8	Wind, N.W., slight, snow on 1st and 2nd, rain on 5th
" 12....	26.25	1,439	54.8	6.5	Wind, S., moderate
" 19....	3	....	....	4.5	" S.E., slight, experiments made between showers, rain fell immediately after volumetric was finished and plate could not be exposed, rain ceased next day, 20th, and a gale commenced. Plate was exposed on 21st during gale

Date of experiment	Number in 10 litres	Number falling per sq. foot per min.	Ratio of the number falling per sq. foot per min. to the number in 10 litres	Temperature in degrees Centigrade	Remarks upon the weather
Oct. 21....	....	8,440	....	4.5	Wind, S., gale
" 27....	12.5	47	3.7	1.0	" N., moderate, experiment made 30 hours after rain
Nov. 2....	27	399	18.4	1.5	Wind, S., moderate
" 10....	0.0	36	higher than 36	—4	" W., moderate, after 2 days of snow
" 15....	2.2	58	26.3	+5	Wind, N.W., moderate
" 23....	4.25	64	15.5	—4	" N.E., moderate, starting to freeze after a warm week
Dec. 1....	0.725	81	108	—21	Wind, N., moderate, blizzard on previous day
" 7....	0.0	37	higher than 37	—8	Wind, W., slight
" 15....	0.19	8.7	45.7	—6.5	" W., slight
" 22....	0.3	38	126.6	—11	" S.E., moderate
" 29....	0.2	8.7	43.5	—8	" S.E., slight
1909					
Jan. 7....	0.2	3.1	15.5	—34	" N., moderate
" 14....	0.2	13	65	—21	" W., moderate
" 21....	1.0	22	22	—8	" E., moderate, no snow for 2 weeks
" 28....	1.0	18	18	—22	" N., strong, little snow since last experiment
Feb. 5....	0.6	23	38.5	—8	Wind, W., moderate
" 12....	0.4	3.5	8.7	—31	" W., moderate, experiment made shortly after fall of snow
" 19....	0.13	5	38.4	—24	Wind, N., moderate
" 26....	0.2	8.7	43.5	—8	" S.E., slight
March 6....	0.3	7.2	24	—7	" N.W., moderate
" 12....	1.0	23	23	—8	" S.E., moderate
" 17....	0.8	21	26.2	—7	" S.E., strong
" 25....	0.0	3	higher than 3	+2	" S., moderate, first thaw following a day of snow
April 1....	1.0	140	140	+2.5	Wind, S., strong
" 9....	2	425	212.5	0.0	" S., strong
" 16....	3	298	76	+6.5	" W., moderate
" 24....	11	2,309	209.9	—4.5	" N.W., strong, gale on previous day

The results of the volumetric experiments have been plotted in Plate 1. The thick continuous line joins the points indicating the results of each experiment. A second line gives the monthly averages. Two other lines give the temperature at the time of each experiment and the monthly average temperatures respectively. The results of the plate method are given in Plate II. The thick continuous line joins the points of each individual experiment. A second line gives the monthly averages. Two other lines give the temperatures at the time of each experiment and the monthly average temperatures respectively.

An examination of the two plates shows that during the winter months, *i.e.*, including the period from about the end of the first week of November to the end of the first week in April, the air in Winnipeg is remarkably free from micro-organisms. The average number in 10 litres during these five winter months is less than one. During the same period in England, as is shown by Frankland's curve,[1] the average number in 10 litres is about 15. The paucity of micro-organisms in the air of Winnipeg during the winter is accounted for by the fact that frost is continuous and therefore prevents micro-organisms from multiplying, also that the successive falls of snow cover up the dust on the ground and prevent its being carried into the air by the wind.

During what may be roughly called the summer half of the year, *i.e.*, from about the end of the first week in April to the end of the first week in November, the average number of micro-organisms in 10 litres was found to be 40.1. The number remains fairly constant during the five winter months, commences to rise in March, increases steadily until the middle of September, and then declines rapidly with the advent of early frosts.

The maximum number of micro-organisms in 10 litres of air, namely 263, was observed on September 28th, 1908. The next highest was 137, on August 24th, and the third highest 100, on September 21st.

The minimum number was found on March 25th, 1909, when 50 litres of air did not yield a single colony. Other low records were as follow: on February 19th, when one Pencillium plant was obtained from 75 litres of air; on December 15th. when one Penicillium plant was obtained from 55 litres; and on December 29th, 1908, and January 7th and 14th, 1909, when on each occasion two micro-organisms were found in 100 litres.

The average number of micro-organisms falling per square foot per minute, from the end of the first week of November to the end of

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[1] P. F. Frankland. Further Experiments on the Distribution of Micro-organisms in Air (by Hesse's Method). Proc. of the Roy. Soc., Vol. 42, 1887, Pl. 3.

the first week in April, was 19.5. The average number from the end of the first week in April to the end of the first week in November was 1,206.6. The highest monthly average was in October, and the next highest in April. Both of these averages coincided with the windiest periods of the year. The minimum number of micro-organisms falling per square foot per minute, namely 3, was observed on two occasions, January 7th and March 25th respectively. The next lowest record, of 3.5, was for February 12th. On February 19th the number was 5.

We may now discuss the effect of the weather on the results. The variation in the number of micro-organisms in the air is due, as has been explained by many other observers, to changes in temperature, alteration in the velocity of the wind, and to the fall of rain and snow. An inspection of the volumetric results plotted in Plate I, shows that temperature has the most influence, for the monthly averages are lowest during the cold winter months, rise steadily during the spring, and are highest during the hot summer months. Showers of rain or snow temporarily clean the air by carrying much of the suspended dust to the ground. This is shown by the following observations. On August 31st, after two rainy days, 12.5 micro-organisms were contained in 10 litres of air, whereas during the previous week, when the velocity of the wind and the temperature were almost exactly the same, the number was found to be 137. The plate method also showed a fall in the number for the same period. On November 10th, after two days' snow, 24 litres of air did not yield a single colony, and only 36 micro-organisms fell per square foot per minute; whereas a week earlier 27 micro-organisms occurred in 10 litres, and 399 fell per square foot per minute. The influence of the wind factor became obvious on several occasions. With an increase in the wind velocity, the number of micro-organisms usually increased, and with a decrease in wind velocity the number of micro-organisms in the air decreased; thus, on April 13th, 1908, there were 30 in 10 litres during a gale, whereas on April 6th, when the wind was only strong, there were 6, and on April 22nd, when the wind was slight, there were 22.5. The effect of wind on the number of micro-organisms falling per square foot per minute is very obvious; thus, during three gales, on April 10th, April 13th, and on October 21st, the enormous totals of 4,180, 8,506 and 8,440 were obtained respectively.

The nature of the Micro-organisms.—The micro-organisms which developed in the cultures were included in the three groups, Bacteria, Yeasts and Fungi. Usually the bacteria predominated; but this was not always the case. Thus on October 6th a pink yeast was a dominant form, there being four yeast colonies to three of other micro-organisms. This yeast came up in the cultures almost every week in the year,

but as a rule it formed only two or three colonies. In the winter, fungus mycelia often developed without any accompanying bacteria.

Up to the present we have not paid any special attention to the species of the micro-organisms. However, the bacteria flora of Winnipeg is extremely varied. This is shown by the differences in colour and modes of growth of the bacterial colonies. Both pink and white yeasts are frequent. Among the fungi we made out the following species: *Cephalothecium roseum*, *Stysanus stemonites*, *Macrosporium cladosporioides*, *Mucor racemosus*, *M. mucedo*, *M. stolonifer*, *Pencillium glaucum* and *Aspergillus glaucus*. There were several other species which, on account of sterility, we were unable to identify. One of these, which was not infrequent, produced a colourless mycelium without spores, but had the peculiarity of turning the gelatine a blood-red colour.

Experiments at St. Charles.

As has already been stated, St. Charles is a small village six miles west of the centre of the city of Winnipeg. The exact place where the experiments were made was a piece of bare prairie at a considerable distance from any human habitations. A single experiment by the volumetric pump method was made monthly during the summer and at intervals of six weeks during the winter. The following is a tabulated statement of the results. For the sake of comparison, the results of experiments made in Winnipeg on some of the same days have been added.

Month.	Number in 10 litres at St. Charles	Number in 10 litres at Winnipeg.
June	15	40
July	8	18
August	14	62
September	9	30
October	12	..
November	3	..
December	0	..
February	0	..
April	2	3
May	4	24

It thus appears that there are about three times as many organisms in the air of the City of Winnipeg as there are out on the prairie six miles away. This can be readily understood when one thinks of the

dust and dirt which are inevitably associated with the streets of a big city, where street-cars, automobiles and other agencies keep the air in constant motion and full of suspended particles.

Comparison of the Volumetric and Plate Methods.

In the course of the year's work altogether, 57 double experiments were made with the volumetric and plate methods on the same days. The plates were exposed immediately after the aspiration of 10 or more litres of air through the experimental tube. The ratio of the number of micro-organisms falling per square foot per minute to the number contained in 10 litres is given in the fourth column of the Table. The average ratio of 54 of the experiments[1] was found to be 45.48, and the ratio varied from 2.9 to 283.

The great variability in the ratio goes to show that the two methods have different values for estimating the number of micro-organisms in the air. The results given by one method are affected by changes in the weather in a different manner to the results given by the other method. Thus, during strong gales, the ratio tends to reach its maximum, as is shown by the data given in the Table for April 13th, 1908, and April 24th, 1909. The lowest ratio, namely 2.9, on September 28th, 1908, occurred when the number in 10 litres of air reached its highest value and when the wind was only moderate. In general, it is not easy to trace the exact causes of the variations in the ratio.

Summary.

The number of micro-organisms in the air of Winnipeg varies from week to week throughout the year. The variations depend, as in other localities, upon temperature, wind, and the fall of rain and snow.

The average number of micro-organisms in 10 litres of air, as the result of 52 weekly observations, was found to be 24. So far as the organic contents of the air are concerned, the year may be divided into two parts—the winter period of five months from the end of the first week of November to the end of the first week in April, during which the average number of micro-organisms in 10 litres is 0.7, and the summer period of seven months, from the end of the first week in April to the end of the first week of November, during which the average is 40.1. The remarkable paucity of micro-organisms in the air during the five winter months is to be accounted for by the continuous frost and the repeated falls of snow, which prevent dust particles

[1] The ratios for November 10th and December 1st, 1908, and for March 25th, 1909, could not be taken into account in obtaining this average, owing to their being indefinite.

of all kinds from being carried into the air. In so far as fewness of micro-organisms is a factor in deciding the hygienic value of the air, Winnipeg must be one of the healthiest cities in the world.

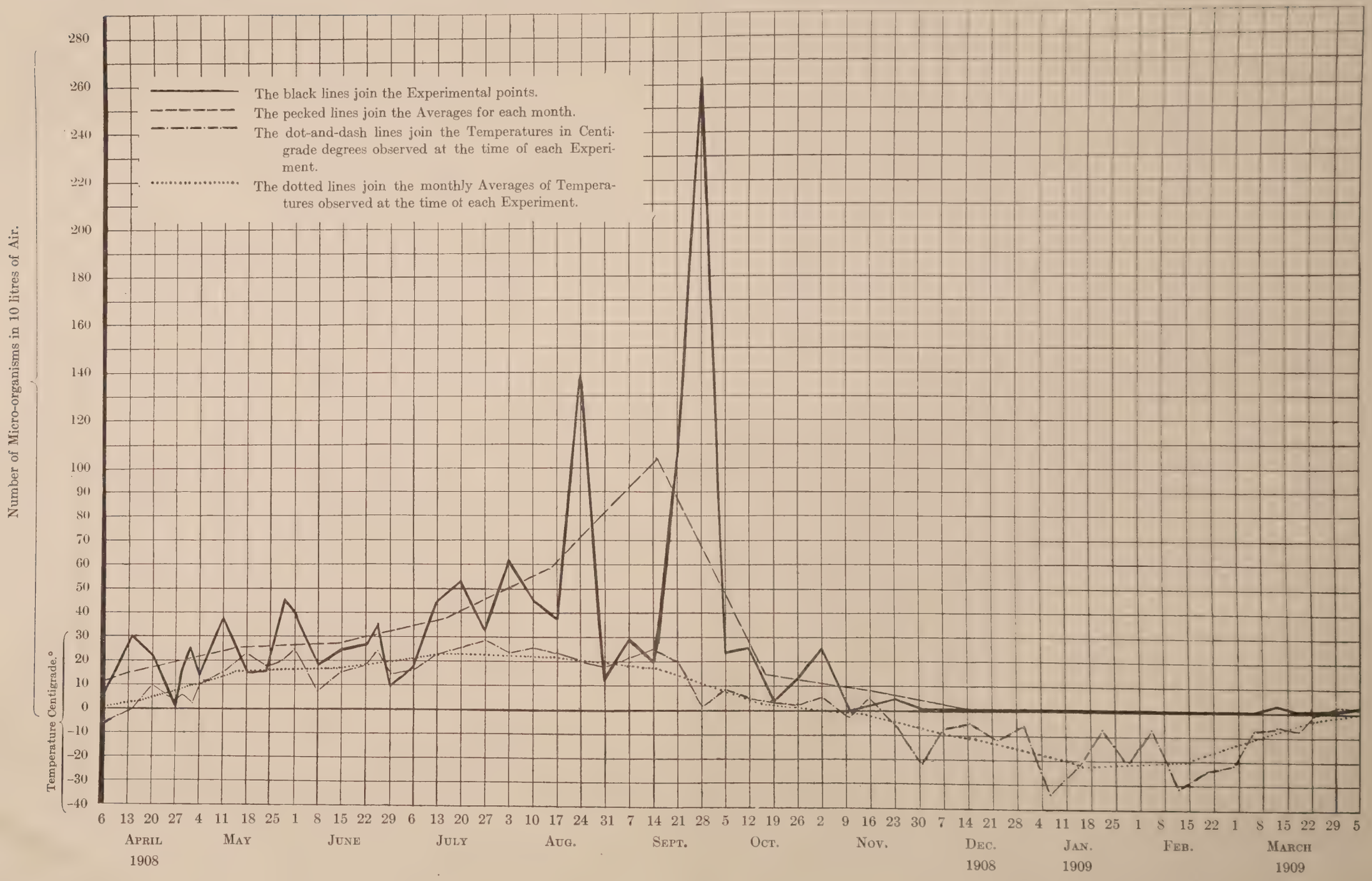
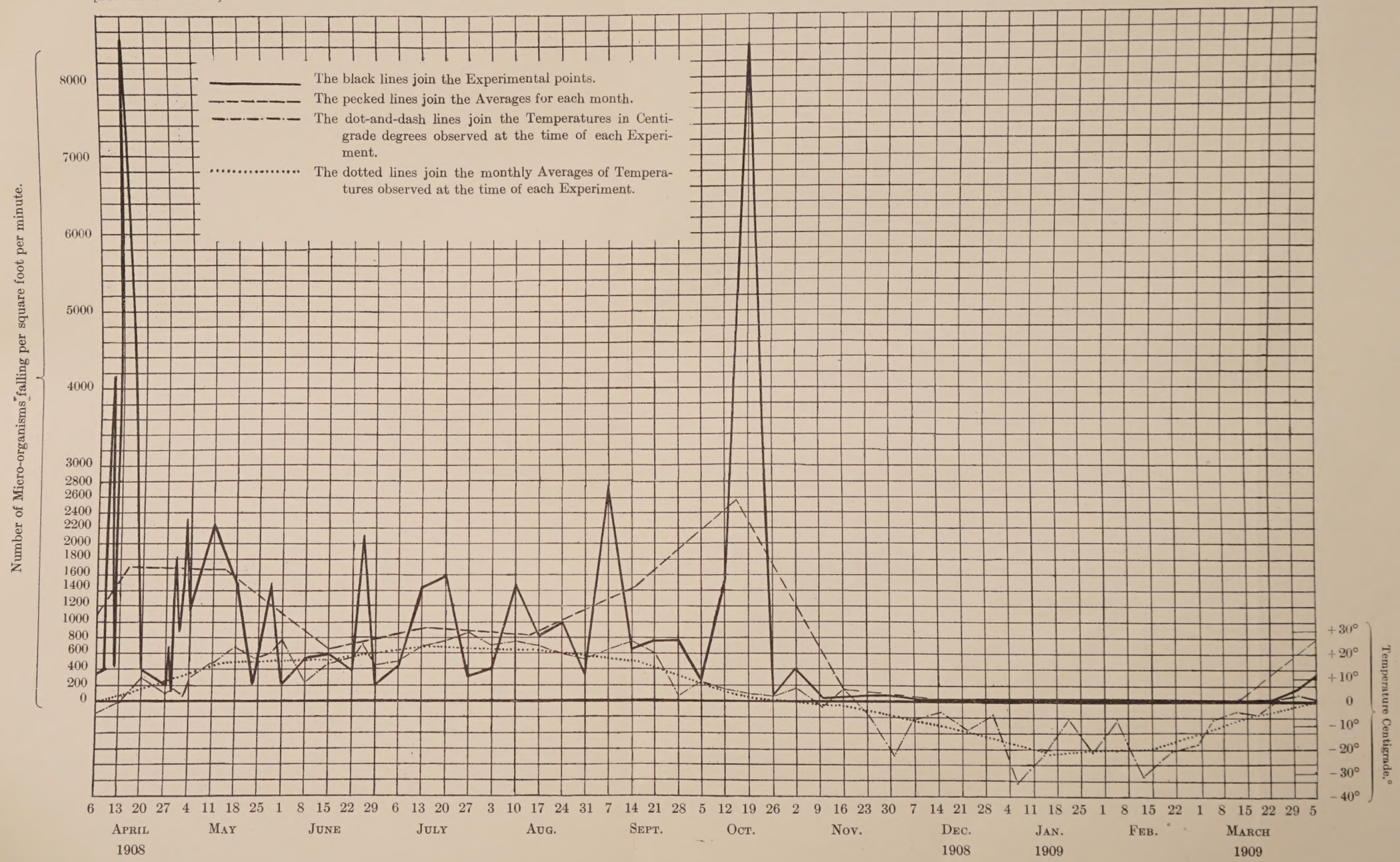
The highest number of micro-organisms found in 10 litres was 263 on September 28th, 1908. The lowest numbers were 0 in 50 litres on March 25th, 1909, and 1 in 75 litres on February 19th, 1909.

The highest number of micro-organisms falling per square foot per minute was 8,506 during a gale on April 13th, 1908. The lowest number was 3, which was registered on two occasions, once on January 7th, 1909, and again on March 25th in the same year.

Comparative experiments made on the same days on the bare prairie at St. Charles, a place six miles from the City of Winnipeg, and in the centre of Winnipeg showed that the air in the town was about three times richer in micro-organisms than that in the country.

The average ratio of the number of micro-organisms falling per square foot per minute to the number contained in 10 litres of air for 54 experiments made during the year was found to be 45.48, and the ratio varied from 2.9 to 283. From the great variability of the ratio we may conclude that the volumetric and plate methods each has its own particular and distinct value for estimating the number of micro-organisms in the air. A curve plotted from the results given by the one method does not coincide with the curve plotted from the results given by the other. Variability in external conditions does not affect the results given by the two methods in exactly the same manner.

AIR COLLECTED ON THE CAMPUS OF THE UNIVERSITY OF MANITOBA, WINNIPEG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